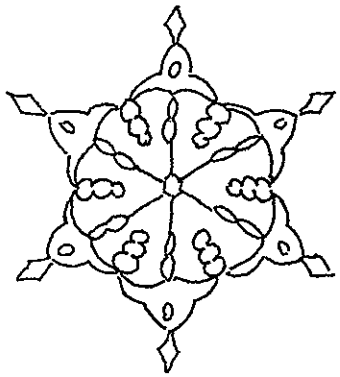

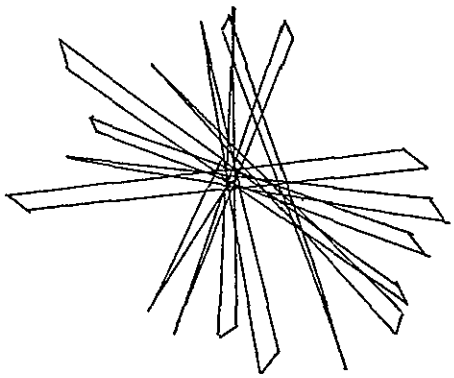

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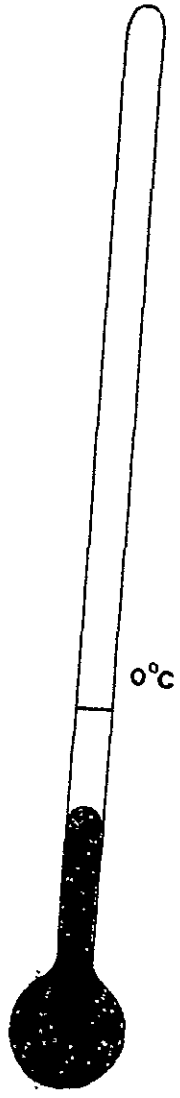

REPORT OF THE INTERNATIONAL POTATO CENTER'S PLANNING CONFERENCE ON COLD HARDINESS

INTERNATIONAL POTATO CENTRE INTERNACIONAL DE LA PAPA

LIMA - PERU

INTERNATIONAL POTATO CENTER

REPORT OF THE

PLANNING CONFERENCE

ON

COLD HARDINESS

Held at CIP - Lima, Peru

February 25-27, 1974

C O N T E N T S

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PLANNING CONFERENCE ON COLD RESISTANCE

Held at CIP, Lima, February, 1974

At the request of Dr. Richard L. Sawyer, Director General of "El Centro Internacional de la Papa" (CIP), a Planning Conference was held to examine priorities and recommend programmes for the next five years to improve the resistance of potatoes to cold.

This report summarizes the discussions and recommendations indicating research priorities and suggesting sources from whom cooperation might be sought to successfully accomplish the envisioned goal of increasing cold tolerance in the potato.

Participants in the Planning Conference were:

C. J. Weiser	Head, Department of Horticulture Oregon State University Corvallis, Oregon, 97331 U.S.A.
L. V. Gusta	Crop Science Department University of Saskatechewan Saskatoon, Sask. 57N 0W0 CANADA
M. J. Burke	Laboratory of Plant Hardiness Department of Horticultural Science University of Minnesota St. Paul, Minn. 55101 U.S.A.

It is with regret that two invited participants were unable to attend: Prof. D. Fenson, Head, Department of Biology, Mount Allison University, Sackville, New Brunswick, Canada. Dr. Krasavtsev, Timiryazev, Institute of Plant Physiology, Botanichezkaya Street, Moscow, 127273. U.S.S.R.

CIP personnel in attending at the Conference were:

K. Sayre	Head, Department of Physiology CIP, Lima
P.H. Li	On sabbatical leave from the Laboratory of Plant Hardiness Deparment of Horticultural Science University of Minnesota
N. Estrada	Department of Breeding and Genetics CIP, Lima
F. Ezeta	Department of Physiology CIP, Lima
W. Roca	Department of Physiology CIP, Lima
O.T. Page	Director of Research CIP, Lima

A position paper entitled: "A Review of Potato and Herbaceous Plant Hardiness" compiled for the Conference by Drs. L.V. Gusta and M.J. Burke is appended (Appendix I).

C E N T R O I N T E R N A C I O N A L D E L A P A P A

PLANNING CONFERENCE ON COLD RESISTANCE

I. AGENDA

Monday, February 25

9:15	Introduction of Participants
	Overview of Objectives of the Planning Conference Dr. R.L. Sawyer, Director General
9:45	General Comments on Position Paper Drs. M.J. Burke and L. V. Gusta
10:00	Coffee
10:15	I Biochemical Considerations of Frost Tolerance Dr. L. V. Gusta
	a) Membranes b) Protein: nucleic acids c) Carbohydrates d) Plant and growth substances
12:00	Lunch at La Molina

Monday, Afternoon

1:30	II Biophysical Considerations of Frost Tolerance Dr. M.J. Burke
3:00	Coffee
3:15 - 4:15	III Theories to Explain Frost Tolerance Drs. Burke, Gusta

a) The sulfhydryl hypothesis
b) The second supercooling point hypothesis
c) The vital water exotherm hypothesis

Tuesday, February 26

8:45	IV Breeding for Frost Tolerance Dr. N. Estrada
9:15	Discussions of Breeding Approach
10:00	Coffee
10:15	V Methods of Determining Frost Tolerance Dr. C.J. Weiser A. Biological Methods 1. Whole plant tests 2. Cut leaf tests 3. Viability tests a) Neutral red b) Conductivity method c) Plasmolysis technique d) Triphenyl tetrazolium chloride test
12:00	Lunch at La Molina
1:30	Methods of Determining Frost Tolerance continued. B. Physical Methods: 1. Nuclear magnetic resonance 2. Thermal analysis 3. Electric resistance
3:00	Coffee
3:15 -5:00	Preparation of Summary Report
7:30	Completion of Summary Report

Wednesday, February 27

9:00	Discussion and Recommendation of Methods of Determining Frost Tolerance Applicable to CIP
	A. Biological Methods:
	1. Whole plant tests 2. Cut leaf tests 3. Viability tests
10:15	Coffee
10:30	B. Physical Methods
12:15	Lunch at La Molina
1:30	Discussion of Breeding Program and Recommendations
	A. Definition of Objectives B. Selection of clonal material C. Facilities for testing Frost Tolerance
3:15	Coffee
3:30 -4:30	General Discussion - CIP Physiology Program Dr. K. Sayre

II. INTRODUCTION

Environmental factors such as excesses of cold, solar radiation, soil dryness and soil wetness, relative to the potato plant, may result in stress responses which reduce yield and quality. While developing countries may have areas in which one or several environmented factors may cause stress during a growing season, it was the intent of the Planning Conference to concentrate on responses of the potato plant to temperature in the range 0 to 6 C. By improving cold hardiness it is anticipated that potatoes can be grown at higher altitudes thus expanding the areas in which potatoes can be profitably produced in Andean countries, Kenya and India.

Freezing temperatures might occur only at the beginning and end of a growing season, or they may occur sporadically throughout a season. In the former case varieties of potatoes which mature in 90 - 110 days may escape frosts. In the latter case, maximum cold hardiness obtained through diligent breeding offers the best hope for either increased resistance to cold or recovery from frost injury.

Injury from air drainage frosts and risk of injury from rapid thawing of frozen plants may be minimized through careful site selection using present technology.

III. PHYSICAL AND CHEMICAL FACTORS RELATING TO COLD HARDINESS

It was generally agreed that Solanum species do not acclimate to low temperatures. Their ability to withstand cold is an inherent ability which does not change markedly during the growing season. Although there may be biochemical markers of frost tolerance, such as enzymes, direct determination of the frost killing temperature is considered the favored approach. The range of cold hardiness for the Solanum species discussed was between - 2°C and 6°C. Both S. tuberosum and S. acaule are truly frost tolerant and can withstand freezing of as much as 50% of the tissue water without

serious injury. Supercooling in potato plants was not considered an effective frost survival mechanism. This tolerance to freezing was discussed in terms of the tissue membranes, proteins, carbohydrates, etc. As is pointed out in the background paper these substances have been implicated in frost tolerance in other plants.

A. Membranes: As many plants acclimate to low temperature, increases in the degree of unsaturation in fatty acids become apparent. This may be important for cold tolerance because an increase in unsaturation makes the membranes more fluid, reduces the probability of a lipid phase transition and makes the membranes more permeable to water.

B. Proteins and carbohydrates: Changes in both of these substances also occur when plants acclimate to low temperatures. Their involvement in frost tolerance is less well understood than membranes. In the case of proteins their importance seems to revolve about their ability to withstand denaturation in the condensed protoplasm of a frozen tissue. Factors of importance here are stability to changes of pH, ionic strength, close association, etc. Low molecular weight carbohydrates may be of importance in protecting proteins and membranes, in controlling the osmotic potential of the cell and in altering the growth patterns of ice as described by Olien. In cases where plants do acclimate to low temperature, the timing of carbohydrates alterations does not correspond directly to the onset of cold acclimation, and therefore, this alteration alone does not appear to be the triggering mechanism for cold hardiness. Plant growth substances are important in the timing of cold acclimation in plants which can acclimate to low temperature; however, potatoes do not seem to be able to undergo cold acclimation.

Various theories have been developed to explain cold hardiness. Three were discussed; the sulfhydryl hypothesis of Levitt, the second super-cooling point hypothesis of Tumanov and Krasavtsev and the vital water exotherm hypothesis of Weiser. All three of these hypothesis deal with the problems arising from protoplasmic dehydration and are discussed in the background paper.

As a cell is frozen first extracellular ice is formed. Continued freezing of water must involve intracellular freezing of water which is

always fatal to the cell or removal of liquid water from the cell which is then frozen in the extracellular region. This latter extracellular freezing is not always injurious to plant tissue and in fact is responsible for survival in hardy plants. The sulfhydryl hypothesis recognizes the importance of cell dehydration as a mechanism, of avoiding intracellular ice. The sulfhydral hypothesis also recognizes the harmful effects of cell dehyration. The condensed protoplasm will probably have altered ionic strength, pH, close macromolecular contacts, and at the maximum, the remaining unfrozen water will be in thin films no thicker than several monolayers. Levitt suggested that the above conditions are conducive to the oxidation of sulfhydryl groups on adjacent macromolecules to form disulfide bridges, cross-linking and irreversible denaturation of macromolecules. The fact that the presence of reducing agents which inhibit disulfide bridge formation prevents cellular injury by frost supports this hypothesis. However, disulfide bridge formation need not be the only source of protein cross-linking and denaturation. Hydrogen bonding between adjacent proteins can lead to similar results and frequently even the most careful protein precipitations are irreversible processes when protein cross-linking by disulfide bridges and hydrogen bonds are not involved. The major point to be obtained from the sulfhydryl hypothesis is that in the condensed protoplasm of frozen tissues macromolecular aggregation is the major factor leading to injury.

The vital water exotherm hypothesis and the second supercooling point hypothesis were both proposed to explain low temperature exothermic events observed at the killing point of woody tissues. Although such exotherms are not observed in Solanum species, the considerations leading to the proposal of these two hypothesis are relevant in the discussion of potato frost tolearance.

The vital water exotherm hypothesis recognizes the importance of water in the maintenance of macromolecular structure. Protein crystals themselves often contain more than 50% water and removal of the water leads to the destruction of the crystals and often to the denaturation of the component proteins. In frozen tissue there is a competition between extracellular ice and intracellular substances for liquid water. These substances include proteins. At the killing temperature the water necessary for macromolecular stability is removed from the cell leading to the irreversible denaturation of the unstable macromolecules. This hypothesis can accomodate, but does not require macromolecular aggregation.

The second supercooling point during the freezing of a tissue the movement of liquid water from the cell becomes restricted due to a sudden change in the membrane permeability. The intracellular water there supercools to below its freezing point before freezing; the intracellular freezing kills the cell. These hypotheses all involve the transport of water from the cell during extracellular freezing and are not mutually exclusive.

Factors only peripherially related to frost hardiness may be of importance in reducing yield losses. Two considerations which surfaced were the development of early maturing potato varieties for the Andean region which could be grown in periods of low frost probability. Regrowth ability in frost-injured plants could also be of considerable importance in reducing yield losses resulting from frosts.

Preliminary results on the freezing process in S. acaule and S. tuberosum indicate that approximately 50% of the leaf water is frozen at the killing temperature. In S. acaule 50% of the leaf-water was frozen at -5.5°C and in S. tuberosum 50% of the tissue water froze at -2.5°C. Other Solanum species being investigated are S. multidissectum, S. chomatophilum, S. bukasovii and S. commersonii. Winter wheats are killed when 87% of their leaf water is frozen (Appendix I)

Rapid methods for screening the frost hardiness of plants have been the limiting factor in breeding for frost resistance. Top prioirty must be given to rapid screening techniques. Screening techniques are of two general categories, whole plant tests and cut leaf tests. The survival of the whole plant in the field is the final desired result. A limitation of the whole plant test in the field is the absence of climate control. Whole plant tests in the growth chamber provide temperature control but some care must be exercised to make sure there is a uniform temperature in the freezing chamber and that roots and other plant parts are not subjected to the cold stress. Correlation between the cut leaf killing temperature and the whole plant killing temperature is very good. An advantage in the cut leaf test is that the plants do not have to be sacrified and can be used for other tests.

IV. SEQUENTIAL TESTING PROCEDURES

Laboratory screening techniques for frost resistance must consider the different types of frost which occur in the field. The two major types of frosts which may occur are from air drainage and radiation frosts. Other environmental factors which may influence the degree of injury are moisture content of the soil and the relative humidity of the air.

Field tests should be designed to take in the above factors and the results obtained in the field compared with artificial freeze tests. Good relative agreement of artificial freeze tests with field tests is essential. Knowledge of the factors which influence survival in the field will permit tailoring of the artificial freeze tests. Field tests should be limited; they ensure validity of the artificial freeze tests.

A. Field Survival Tests

1. The influence of air drainage. Test sites could be located on the side of a hill with a gentle slope. Genotypes with a known range of frost tolerance would be planted along the hill. Since the lowest part of the hill would be the coldest a gradient effect would be created due to air drainage. Temperature recording devices should be used to monitor the temperature at different elevations. Following a frost the plants would be evaluated for injury. Results from this study would then be compared to artificial freeze tests.

2. Radiation Frosts. Radiation frosts occur on clear cold nights where there is little or no movement of air. This results in conditions where the temperature of the leaves is lower than the surrounding air. Test sites should be selected at the higher elevations where conditions would favor this occurrence. Replicated trials with genotypes of known hardiness would be planted out. Following a radiation frost the plants would be rated for survival and the results compared to artificial freeze tests. Ideally it would be of benefit to know both, the leaf and air temperature during a frost.

3. East vs. West Slope Planting. There is evidence to indicate that potatoes planted on the east slope of a hill are injured to a greater degree following a frost than potatoes located on the west slope. This is thought to be due to the rapid thawing of frozen plants by the morning sun. The rate of thawing may be then another factor in addition to low temperature that affects survival and should be considered in the artificial freeze. A series of shading experiments for plants located on the east side of a hill would help resolve this question.

4. The Influence of Maturity. There is little evidence on whether maturity has an effect on the hardiness of potatoes. A date of planting experiment could be initiated in an area where frosts are predictable for a given month. Genotypes of a known range of hardiness could be planted every second or third week up to the time when a frost usually occurs. This would establish a series of plants at different stages of maturity. Following a frost the plant would then be rated for injury.

In all of the above field tests, temperature recording devices should be located at the test sites to provide information on the minimum temperature, the duration of the minimum temperature, and the rate of temperature change.

5. Collection of Hardy Genotypes. A survey of farmer's fields in the high altitude areas following severe frosts may be one means of selecting very hardy genotypes. Many of these potato fields do not consist of pure lines and also some of the potatoes are from very old lines. These older lines may be potentially more frost hardy since they have been handed down from generation to generation. Genotypes which clearly show superior hardiness should be collected and tested for cold hardiness by artificial freeze tests at the center.

B. Artificial Freeze Tests

Artificial freeze tests have the advantage of precision in temperature control, flexibility in regard to the desired temperature and the experiments may be replicated in time. A standard freeze test would permit comparison of results obtained by different researchers.

1. Immediate Considerations. A low temperature water bath is available at the Center now which would permit immediate screening of genotype. Sukumaran has developed an artificial screening technique using the cut leaf test for potatoes. One test temperature e.g. - 4°C would be suitable initially for screening populations and quickly removing the non-hardy genotypes. Plants could be nucleated at -1.5°C and rated for damage either visually or by conductivities. Modifications should be carried out to shorten the time required for the freeze test. Care should be given to the avoidance of sample desiccation during preparation.

2. Short Range Considerations.

a) Whole Plant Freeze Tests. A freezing-test chamber will be available at the Center shortly which would be suitable for freezing whole plants either in flats or in pots. Dr. N.Estrada is familiar with this technique and has published on it. The freezing test chamber could be used to compare the results obtained from the cut leaft tests. There is evidence that there is agreement between the cut leaf test and whole plant test. In the whole plant test the complete plant may be sacrified; however, if the plants are generated from cut seed pieces or tubers the breeding stock may not necessarily be lost. Seedling stock, however, may be lost. This chamber may also be used to evaluate regrowth following a freezing stress. This information would be of value when plants are exposed to injurious frosts early in their development.

The whole plant freeze test may also be used to determine the effect of repeated frosts on survival. Plants which survive a single frost of -4°C may be injured by a subsequent frost of -2 to -3°C. Repeated frosts in the field are not uncommon and their effects should be considered in artificial freeze tests.

The whole-plant freeze test could be used to determine if compounds are translocated from injured leaves to the remaining plant parts which have an effect on subsequent effect on regrowth and recovery.

An extension of the program would be the incorporation of a modified domestic deep-freeze. The conversion of domestic deep freezers has been described in the Canadian Journal of Plant Science. This type of freezer offers a finer control over temperature as compared to commercial growth chamber. In addition to using it for whole plants it may also be modified for use with

the cut leaf test. A fan would be installed in the test chamber for maintaining a uniform temperature.

b) Temperature Gradient Bar. The construction of a temperature gradient bar would facilitate the screening of large populations. This method would be of value in determining the hardiness of genotypes within one degree. Other experiments of value which could be done are to determine the effect of frost duration on survival and the effect of the rate of temperature change on survival.

c) Viability Tests. At present the simplest test for evaluating frost injury is the visual test. It is rapid and cheap and agrees with field observations. Other methods used for estimating injury are laborious, slow and costly. The conductivity method is relatively simple and quick and provides a good objective test for rating injury.

d) Frost Hardiness Rating of Climate Races. Within a given race there may be considerable differences in frost hardiness. A good example of this is S. acaule which has been reported in the literature to withstand a range of temperature from -4 to -9°C. Knowledge of the variation on hardy genotype would be essential in a breeding program and may also be of benefit in elucidating the mechanism of frost hardiness. The most cold hardy races would be the material of choice in breeding programs. Climatic races should be collected from a range of habitats and then evaluated for cold hardiness by artificial freeze tests. A series of test temperatures should be employed to determine the hardiness within one degree. Once the hardiness has been assessed the plants could be maintained in the germ plasm collection for future reference.

e) Reference Table for Frost Hardiness. Since the Center has the best collection of potato germ plasm in the world, a reference table could be established for frost hardiness. Genotypes could be screened for hardiness under controlled conditions and then ranked in order of hardiness. This information would be of considerable value for plant breeders around the world which have a frost problem with potatoes.

f) Effect of Moisture on Cold Hardiness. The water status of the plant has been shown to have an effect on cold hardiness. In irrigated areas a study may be initiated to withhold water during critical cold periods to determine the effect on cold survival. Follow up tests could also be conducted in the laboratory.

Long Term Considerations

The ultimate screening technique in screening for frost tolerance should be simple, quick and non-destructive. Although such a method does not exist at present, perhaps as we gain more knowledge on the freezing process the ultimate test will be developed.

With the establishment of a breeding program for frost hardiness other factors such as photoperiodic insensitivity (day neutral with regard to tuberization), disease and insect resistance may have to be evaluated to determine how these are inherited along with frost hardiness. The determination of the mode of frost hardiness inheritance could greatly facilitate an effective breeding program. It is still not known if hardiness is quantitatively or qualitatively inherited, whether maternal inheritance is a significant factor, or whether extensive epistasis offers potential for telescoping breeding cycles. Resolution of these and related genetic questions could increase the effectiveness of breeding efforts and shorten the time required to develop frost resistant cultivars with the desirable characteristics.

There is some evidence to indicate the number of stomata may be involved in leaf frost hardiness. Once this observation has been established the mechanism should be studied if there is a positive correlation.

V. RECOMMENDATIONS - PREAMBLE

A. Considerations in Arriving at Recommendations:

1. What is needed to avoid or significantly attenuate losses due to frost injury in potato? (an idealized projection objectives).

2. What role can and/or should the Center play in meeting these objectives? (unique strengths of the Centers and capabilities for providing international leadership and coordination to such a program in ways which are commensurate with overall charge and objectives of the Center).

3. What can likely be achieved in reducing freezing damage and losses to potato. (Realistic assessment and identification of priorities for immediate (1-2 years); short range (3-5 years) and long range (5-10 years) goals).

In making recommendations the conferees have attempted to take into account the considerations listed above. Insofar as possible the planning process involved sequential consideration of these points in the order they are presented; e.g. idealistically what would be the ultimate solution(s) -- What are the unique capabilities of the Center in achieving such solutions? -- Why is it realistic to undertake and in what order?

Since time, resources, and manpower are limited for addressing all problems associated with attenuating frost damage to potatoes, (and other important problems) it is necessary to make certain assumptions in arriving at recommendations -- particularly in focusing effort in the immediate and short-range phases of the program. These assumptions are listed in the next section.

As work progresses, and when these assumptions are verified or proved to be invalid, it will be appropriate and necessary to alter the priorities and recommendations accordingly.

B. Assumptions made in Arriving at Recommendations.

1. Frost damage is a major limiting factor to potato production on a world-wide basis; a problem of sufficient magnitude, scope, and impact to warrant a significant effort on the part of CIP.

2. The problem of reducing frost losses on a field scale can be approached in several ways, but breeding frost resistant cultivars provides the best means of achieving that end. (Appendix II).

3. Screening for frost resistance is a relatively simple process compared to screening for other pest, disease, and stresses which limit production.

4. Screening methods now available provide valid means of selecting genotypes which will have field resistance to natural frosts. a) The relative hardiness of excised leaflets reflects accurately the relative resistance of whole plants in the field; b) Avoidance of freezing by supercooling is not a significant factor in frost avoidance under field conditions; c) Potato plants do not acclimate significantly to resist freezing stress; d) The relative hardiness of seedlings reflects the relative hardiness of mature plants grown from tuber seed pieces; e) Relative differences in resistance of young and old leaves to frost are similar among genotypes; f) The nature of freezing resistance in different potato species and genotypes is basically similar and related primarily to the status and amount of unfrozen water in the tissues; g) Leaves are the most frequently injured tissue and appropriate test tissues for assessing field hardiness; h) Freezing resistance is closely correlated with thawing resistance which may be a factor in field damage; i) Repeated exposure to frost is likely to accentuate (amplify) injury.

5. Efficient mass selection procedures can be developed and refined within the next 3 years which will permit efficient screening of large populations.

6. Further elucidation of the nature of freezing stress in the field and the nature of the freezing process in potato leaves is appropriate, and offers the most likely avenue for long-ranged breakthroughs in genetic improvement and/or physiological manipulation of potato to increase resistance and reduce damage caused by freezing and perhaps other stresses (drought, heat, salt) which also involve tissue desiccation.

C. Narrative and Elaboration on Considerations and Assumptions Itemized in A and B.

1. Productive cultivars of potato which resisted -6 °C at all

stages of development would solve many frost problems which now limit production in many parts of the world. The impact of frost resistant cultivar development would be considerably accentuated if frost resistance was incorporated into genotypes which also have a wide range adaptation especially in terms of photoperiodic insensitivity in tuber initiation. Attempts to genetically achieve a combination for these characteristics would be a worthwhile long-range objective.

2. Although a number of wild species of tuber-bearing Solanums posses frost resistance of the magnitude mentioned in the preceeding section there has been little progress in incorporating this characteristic into cultivated varieties. The Center is in a unique position to provide leadership and coordination in the development of frost resistant potato cultivars resistant for several reasons, involving personnel, geographic, and germplasm considerations. There are no insurmountable, or even major, barriers to progress. As described in another section of this report on breeding implications, crossability, heritability, sources of resistance, and other genetic considerations do not limit progress. Valid controlled freezing tests are now also available which with minor refinements can be adapted for mass selection. The missing element which has limited progress in breeding frost resistant potato varieties has been and is a team approach combining breeding and physiological expertise into an effective working unit. Geneticists and pathologists have worked effectively together in breeding for disease resistance, but cryobiology and stress physiology is a specialized field of study which few breeders feel competent to undertake and where few research colleagues have been available to cooperate directly. The critical mass of interdisciplinary expertise provided by the Center staff offers a unique opportunity to overcome this deficiency which has seriously hampered previous programs. Clearly the unpredictability and variability implicit in "test freeze" field selection for frost resistance have made that approach an inappropriate and unproductive way to achieve and sustain an effective mass selection program.

While mass selection for frost resistance in the field has not been effective there are a number of important questions that can only be resolved in the field. The germplasm collection, proximity to highland production areas where frost is a serious problem in the field, access to native species in their natural habitats, access to field testing sites subject to frost and other geographical considerations provide the Center with unique opportunities to effectively evaluate assumptions (Particularly those listed in Section B, item 4) and explore potentials. Appropriate

studies which take advantage of germplasm and geographic features of the Center operation are described in more detail in the recommendations section of this report.

3. Frost problems and opportunities for solving them take several forms. Emphasis in this report are placed heavily on breeding for resistance because the conferees believe that this offers the best chance for significant reduction of losses from freezing in areas where frost is a crucial problem. Physiological and cultural manipulations to reduce frost damage may also arise which warrant attention, but none look particularly promising at this time. In irrigated areas withholding water prior to frost may be worth exploring since highly hydrated tissues are more subject to freezing damage in some crops. Heavy potassium fertilization seems to increase frost resistance in potato, but the practical implications are doubtful. Considerable chemical cryoprotectant work is underway on a variety of crops but to date there has been no striking success and no widespread commercial use of chemical protectants. It would be well to keep a close watch on developments on other crops, but undertaking extensive efforts on potato seem ill-advised unless something promising is identified. Frost problems and possible approaches differ somewhat in different situations: e.g. In:

a) High elevations where frosts cause extensive losses; where frosts can and do occur any month of the year; and where potato is a dietary staple in the farmer's diet.

b) In commercial production areas where frosts at either end of the generally frost-free growing period cause significant losses.

c) In new areas where potatoes are not now grown.

The immediate and short-term recommendations in this report are aimed primarily at situation a) in the preceeding list because frost losses in these situations constitute the most recurrent and serious losses in terms of their social, if not economic, impact. Situation b) probably warrants a different approach-specifically selection for early maturing types which can avoid damage by maturing during the frost-free growing season rather than breeding for resistance per se.

D. 1. There is little data available on the world-wide losses caused by frost on potatoes although it has been said to be the single most limiting factor to production in many areas. This lack of definitive information may be due in part to the acceptance of crop losses from uncontrollable natural causes such as weather. At the high elevations in India, Kenya, Colombia, Ecuador, Bolivia, Perú and elsewhere where frost is a particularly serious problem, much of the production is for home consumption and definitive data on production and losses due to frost are much more difficult to collect than in commercial production areas.

Several points, however, are clear. Fall and spring frost damage is almost a ubiquitous problem wherever potatoes are grown in the temperate zones. In the mountain and high plateau areas of Africa, South America, and the Indian subcontinent where the native people are most dependent on potatoes as a major dietary staple food the problem of frost damage is unfortunately most severe. (See "Prospects for the Potato in the Developing World", pages 27-44, 50-53, 217-224).

2. The major points are: Potato leaves frozen under controlled conditions either survive or are killed; that visual observation of the water-soaked appearance of dead leaves or simple conductivity tests can be used to evaluate injury; there appears to be good correlations between controlled freezing tests and field survival. Compared to screening for other types of pest or disease resistance this is an extremely simple, straight forward procedure. Because of this it seems reasonable to tool up for screening of large populations for frost resistance. Where concurrent breeding programs are undertaken (e.g. for development of frost and nematode-resistant, day-neutral cultivars) it would be reasonable to screen initially for frost resistance, and to screen the much smaller populations remaining for other characteristics which aren't so readily evaluated.

II. The preliminary NMR data which indicates that death in frost resistant or frost susceptible genotypes may occur at the point when 50% of the water is frozen (50% of the water is frozen at different temperatures) suggests that there may be some unifying principles involved in frost injury and resistance in potato. Elucidation of these principles could provide basic information with far-reaching implications for increasing frost, drought, salt and heat resistance of potatoes and other crops; stresses which

all involve resistance, avoidance, or tolerance of protoplasmic dehydration. Frost resistance research on potato could also point the way to similar research on other herbaceous crops which have received little if any attention (e.g. corn).

VI. RECOMMENDATIONS

I. SHORT TERM (1 - 3 years)

1. It is recommended to establish whether the relative hardiness of excised reflects accurately the relative frost hardiness of whole plants in the field:

a) To verify that significant acclimation does not occur so as to invalidate relative cold hardiness ratings;

b) To verify whether the cold hardiness of excised leaflets of varying physiological age reflects the hardiness of whole plants under controlled conditions;

c) To determine whether a correlation exists in the cold hardiness of selected genotypes (with known hardiness range) between laboratory and field hardiness responses.

2. It is recommended to utilize available equipment resources to commence excised leaflet screening tests as soon as possible.

3. It is recommended that equipment such as (I) a thermal gradient bar be developed and perfected, or (II) that multiple thermal baths be purchased to permit rapid assay of cold hardiness in excised leaflets.

4. It is recommended that a search be initiated to locate sites to evaluate cold hardiness under environmental conditions predominantly associated with (I) radiation frost, (II) air-drainage frost, and (III) the influence of solar radiation on thawing.

5. It is recommended that selected freezing phenomena be identified for study at a physiological level through a suitable Contract Project coordinated with the needs of CIP.

II. INTERMEDIATE TERM (2 - 5 years)

1. It is recommended that studies be initiated as early as possible to determine the inheritance of cold hardiness.

2. It is recommended that breeding initiatives consider the integration of selected specific characteristics to be combined with cold hardiness.

3. It is recommended that studies of the physiology of freezing process be phased to provide a continuing program arising from Contract Project findings.

III. LONG TERM (5 - 10 years)

1. It is recommended that the possible interrelationships of resistance to cold, heat, and drought stress be evaluated.

A P P E N D I X I

A REVIEW OF P0TATO AND HERBACEOUS PLANT HARDINESS

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I. INTRODUCTION

The common potato (Solanum tuberosum) is considered to be one of the most important foods in the world and rivals wheat in total value. The center of origin of the potato was in the South American continent with wild tuber-bearing Solanums also occurring in Central America, Mexico and as far north as Colorado (1).

Potatoes are the staple food in the Andean region of South America. Due to the high incidence of frosts, high protein crops such as wheat, rice and corn cannot be grown successfully at the high elevations and even when frosts are no problem, potatoes yield twice as much protein per acre as wheat, rice and corn. It is an excellent dietary source of protein containing all the essential amino acids for human nutrition. Though nearly 80 percent of the tuber is water, its starch and protein content are roughly 20 and 2 percent, respectively (2).

Frost is the major factor limiting potato production in the Andean region of South America. Depending on the intensity of the frost, either the crop may be killed or the foliage damaged, resulting in delayed maturity and reduced yields. In 1967 severe frosts in Colombia resulted in an estimated 50 million dollar loss (3).

Solanum tuberosum, the most commonly grown species in North America, possesses very little of no frost tolerance. A number of Solanum species (S. acaule; S. chomatophilum; S. commersonii; S. x juzepczukii and S. multidissectum) are considered to be very frost tolerant. Attempts to incorporate this character into presently frost sensitive cultivated potatoes have lagged due to incompatibility problems and the lack of necessary screening techniques required for large segregating populations.

The objectives of this review are to discuss known data on the freezing process in plants, discuss several of the theories to explain frost hardiness and to outline some of the commonly used methods for assessing frost hardiness.

II. GENERAL CONSIDERATIONS OF FROST TOLERANCE

The resistance of plants to sub-freezing temperatures has been studied rather intensively for over a century. For recent detailed reviews on this subject, the reader should consult Levitt (4), Olien (5), Alden and Herman (6), Mazur (7), Weiser (8), Parker (9) and Meryman (10). Unfortunately in this case, most of the work has been concerned with the hardiness of woody plants which are extremely hardy. Generally, herbaceous plants cannot withstand temperatures below -20°C. The hardiness mechanism in herbaceous plants may be quite different than that in woody plants. The review of Olien (5) deals extensively with frost tolerance of winter cereals.

In spite of the vast amount of research in this area, it is still not known how freezing kills plants and how plants adapt to freezing stress. In the case of potatoes where the level of frost tolerance is only a few degrees, a reliable and quick method for screening large segregating populations has not yet been developed. This has complicated the problem of determining the inheritance of frost hardiness. Frost tolerance is an inducible genetic system controlled by low temperature. Photoperiod has been shown to be involved in many deciduous woody perennials (11-13) but not for certain woody evergreens (14) or winter cereals(15). The transition from a tender state to the hardy state is a metabolic process which requires energy. Energy is supplied through photosynthesis (5) and in the case of seedlings from energy reserves in the seed (16, 17). The problem of frost tolerance is complicated further by the fact that hardiness is not a static entity but is affected by nutrition, temperature, date and method of seeding, physiological developments, humidity, rate of growth and moisture content of tissue and soil. Although the adaptation process requires considerable time, many herbaceous species deacclimate rapidly upon exposure to warm temperatures.

There is still some dispute whether the Solanum species acclimate to frost. Several authors suggest an acclimation period of two to three weeks of cool temperatures is required to differentiate hardy from non-hardy species (18, 19); whereas other researchers have shown that potatoes have inherent frost resistance and do not further acclimate (20, 21).

In addition to low temperatures, environment may have an effect on frost tolerance in potatoes. Dry conditions (22) and

low humidity (23) have been shown to markedly increase resistance. Similar results have been obtained by Metcalf et.al. (24) in studying frost tolerance of winter cereals. A small increase in moisture content of the tissue can result in a very large difference in plant survival. Hudson and Idle (23) noted that a reduction in turgor of the protoplasts had a marked effect on the freezing process in S. acaule. In tissue with a high moisture content, water has nearly a single freezing point and the resulting ice crystal causes disruption of the tissue (25). Thus, if freezing occurs early in the morning, damage to the tissue would probably be greatest due to the fact that plants would have regained full turgor overnight.

Hudson (26) claimed that one of the limitations of the cut leaf test was the variability in frost resistance in leaves due to their maturity. As the leaves matured, there was a decline in frost tolerance. Mastenbrock (18) also found that the growing tip of potatoes may be killed although there was not any apparent damage to the rest of the plant. In contrast to the above findings other authors have not detected any change in frost resistance with maturity (27, 28).

Other factors that may influence frost tolerance are; etiolation tends to decrease tolerance (26) and viral infection may increase tolerance by one degree (27). A rosette growth habit has been associated with winter survival, but this does not appear to result in an increase in frost tolerance (27). This growth habit may be of benefit in the case of snow cover.

Repeated frosts have an amplifying effect on injury (25). Plants may be slightly injured when subjected to a low non-lethal temperature; however, a subsequent cold treatment of lower intensity may kill the plant in its weakened condition. The ability of a plant to recover from injury is genetically controlled (5). Fully acclimated hardy winter wheat can withstand a slow freeze to -19°C; however, after two refreeze and thaw cycles the crown tissue will only survive - 12°C (29). Undoubtedly repeated frosts in the field will have the same effect on potatoes. Although potato seedlings were relatively unharmed by an initial frost of -2.8°C, Ross and Rowe (30) found that two subsequent frosts of -1.1 and -2.8°C resulted in apparent injury. Hayden, Dionne and Fensom (20), measuring the electrical impedance of stem tissue from S.acaule and S. tuberosum, found a drop in electrical impedance following a slight frost. A drop in electrical impedance suggests ion leakage from cells due to membrane injury.

III. BIOCHEMICAL CONSIDERATIONS OF FROST TOLERANCE

Since frost tolerance is under genetic control, certain genes are turned on during periods of low temperature to bring about frost acclimation. Elucidation of either the genes or enzymes involved may provide one way of tailoring the plant to survive frosts. Identification of key enzymes could enable the breeder to use these as markers in a breeding program. Some chemical changes which occur during cold acclimation are:

A. Membranes.- During freezing, water moves from the protoplasm to the site of ice crystallization due to the free energy difference created by ice. When the energy required for ice to grow into protoplasts becomes less than the energy required to move water out, membrane injury will occur. Normally membranes are considered to be in a fluid state, but at low temperature and depending upon their chemical composition, they may undergo a phase change to a more solid state (31). This will result in a reduction of membrane permeability causing water to become trapped and freeze intercellularly (32).

In many organisms grown at low temperatures, the membrane fatty acid composition is less saturated (33-38). This increases the permeability of the membranes (35) and keeps them in a fluid state at low temperatures (39). Pomeroy, de la Roche and Miller (40), working with winter and spring wheats, reported an increase in the level of unsaturated fatty acids in the mitochondria from all varieties when grown at 2°C. It is not known if the increase in unsaturation is a low temperature growth response or just one aspect of the plants ability to adapt to low temperatures. Undoubtedly, several physiological changes are occurring during cold acclimation and if these do not occur in proper sequence or if one or more is lacking, then the full hardiness potential is not expressed.

Olien (5) has shown that ice penetrates non-hardy protoplast easier than hardy protoplasts. Membranes from hardy tissue are more elastic than membranes from non-hardy plants and therefore are able to withstand the stresses induced by the growing ice crystal.

Phospholipid changes occur during cold acclimation of black locust (41) and alfalfa(36). However, de la Roche, Andrews and Kates (17), using various techniques for phospholipid extraction,

could not detect any difference in phospholipid composition (i.e. phospholipid class) in wheat seedlings grown at 24 and 2°C.

B. Protein and Nucleic Acids.- The role of proteins, either in the catalysis of metabolic reactions or in maintaining the structural organization of membranes, suggests an intimate involvement in the development of frost tolerance. Qualitative (42-44) and quantitative (45-47) changes occur during cold acclimation of many plants. However, other researchers reported no detectable changes in protein (4,48).

Many of the current hypotheses proposed to explain cold resistance and injury in one way or another are centered around the role of proteins and their involvement in the cell's structural integrity. Certain enzymes are known to be cold labile. For example, glycogen phosphorylase (49), lipoxidase (50), D-amino acid oxidase (51), phosphatase and peroxidases (52) undergo reversible partial inactivation in vitro at low temperatures. Irreversible cold inactivation has been shown for frog carbamyl phosphate synthetase (53) and beef mitochondria ATP ase (54). Certain lipoproteins cannot be frozen without denaturation and loss of characteristic solubility properties (55).

Certain proteins have a protective effect during freezing. Williams (56) reported that during cold acclimation of Cornus florida, a glycoprotein is synthesized which is capable of binding large amounts of water. Heber (57) has isolated two proteins from spinach leaves which were more effective than sugars in protecting chloroplasts. DeVries (58) was able to show that certain glycoproteins protected artic fish against freezing injury.

An increase in RNase occurs in leaf tissue subjected to a stress. Cold (59), insect infestation (60), osmotic shock (61) and excision (62) resulted in dramatic increases in RNase activity in a variety of plants. A rapid loss of all RNA species occurs in boxwood leaves subjected to a lethal frost (46). Recent research has indicated cryoinjury in animal cells may result from rapid enzymatic degradation of cell constituents following disruption of intracellular compartmentalization (63). Although RNase may not be directly involved in cold injury, it certainly may have a role in recovery.

Quantitative and qualitative changes in ribonucleic acids occur during cold acclimation of woody plants (41, 46, 47). With the development of the full hardiness potential, there is a marked reduction in rate of metabolism of radioactive nucleic acid

precursors into RNA in barley (64), wheat (65) and boxwood (66) leaves. This is in support of the evidence that growth cessation is required for complete acclimation.

Li and Weiser (67), working with frost sensitive S.tuberosum, reported an increase in all RNA species when the plants were grown under short days and low temperatures. Short day and low temperatures also stimulated an increase of 32P incorporation into all RNA species (68).

Purines and pyrimidines increase cold hardiness, proteins and nucleic acids in alfalfa crowns (69). However, certain purines enhance low temperature breakdown in apples (70).

C. Carbohydrates.- In many plants, sugars increase in the fall as plants acclimate to cold and decrease in the spring during deacclimation (71). This has led many researchers to suggest that sugars have a causal role in cold hardiness (72-74). Also, if non-hardy plants are incubated in sugar solutions, there is generally an increase in frost tolerance (75-77). Sugars have also been shown to protect isolated spinach chloroplasts (74). Steponkus (72) suggested that during hardening an alteration in protein structure increases this affinity for sugars. The bound sugars are then able to protect against protein denaturation during freeze-dehydration.

However, an increase in sugars does not always parallel the increase in frost tolerance (4). Fuchigami, Weiser and Richardson (78) were unable to show an increase in frost tolerance of redosier dogwood with various levels of sugars fed continuously. Also in the case of potato plants, low temperatures increased the concentration of sugars but there was no apparent effect on frost resistance (78).

Xylans, high molecular weight carbohydrates, have a direct effect on ice crystal and on the type of ice structure formed (79). Olien (5) has shown that these xylans compete with water for sites on the growing ice crystal. Sakai (80) has identified several polyhydric alcohols which act as antifreeze agents.

D. Plant Growth Substances.- During cold acclimation there is a cessation of growth or slowing down of growth. Many herbaceous plants adapt a rosette growth habit or appear stunted. These observations suggest that during cold acclimation there is a reduction of growth promoting hormones. Cold tolerance of winter cereals has been

reduced by exogenous application of gibberellic acid (GA_3) (81). Applications of 2-chloroethyl trimethyl ammonium chloride (CCC) to winter wheat induces small increases in cold hardiness (82). Exogenous applications of CCC reduce the gibberellin content of many plants (83-85) by inhibiting certain steps in gibberellin synthesis. This led Roberts (82) to suggest that during cold acclimation of winter wheat there is a reduction of endogenous gibberellins.

Exogenous applications of IAA to winter wheat increased the content of natural auxins, decreased growth inhibitors and stimulated plant growth (86). This resulted in a loss of frost resistance of winter wheat plants grown at 5°but not at 0°C. Cold treatment of winter wheat seedlings resulted in a 10-fold increase in indoleacetic acid oxidase (87). An increase in IAA oxidase would tend to keep the endogenous auxin at a low level and thereby prevent the stimulation of growth and the concomitant loss of cold tolerance.

Abscisic acid (ABA), a natural growth inhibitor, increased the hardiness level of Acer negundo (88). ABA has been implicated in the regulation of water balance in plants by inducing stomatal closure and increasing water permeability (89-92). The content of endogenous ABA was found to be very responsive to water stress (91) and mineral starvation (93). Cytokinins on the other hand appear to act as check on ABA. Evidence suggests that cytokinins tend to open stomata and reduce membrane permeability to water. Both membrane permeability and water content have been implicated in frost tolerance of potatoes.

IV. BIOPHYSICAL CONSIDERATIONS OF FROST TOLERANCE

Upon freezing of tissue, the cell water will supercool if there are no ice nuclation sites. MacKenzie and Rasmussen (94), have shown that cells are not ice nucleators; therefore, ice usually forms in the extra-cellular spaces first. As the extracellular ice crystal grows, water moves from the cell due to the free energy difference created by the ice. As more water is lost the cells start to contract and become dehydrated (4). The shape of the ice crystal depends on the extent of supercooling, and the rate of temperature decline. With slow cooling and no supercooling, amorphous ice crystals are formed which are

considered noninjurious to the cell (4, 5). No two ice crystals have the same shape and different shapes have different effects on the distribution of the plants water and also on the ease with which they penetrate the protoplasm (95). This may help explain the wide range of killing temperature reported for potatoes (26).

Crystallization does not proceed into the protoplasts of hardy cells because the plasmalemma acts as a barrier to ice crystal growth (5, 71). In the case of tender plants, extracellular ice grows through the protoplast, resulting in death of the cells (96). During frost acclimation the plasmalemma undergoes a transition and becomes a barrier to ice growth. However, this is not the only reason why plants become frost tolerant. Olien (25) identified five basic patterns of water redistribution in barley plants during freezing. The water redistribution patterns were dependent on the rate of freezing, the resistance of the protoplasm to ice penetration, the moisture content of the cell, the pattern of initial ice growth in the tissue and various other factors which contribute to the hardiness of the cell. Depending upon the above conditions, Olien was able to demonstrate that freezing occurred either as an equilibrium or non-equilibrium process. Non-equilibrium freezing was associated with tender or semi-tender plants. Sukumaran and Weiser (97) were able to show that freezing occurred as a non-equilibrium process in S. tuberosum and as a semi-equilibrium process in S. acaule. From the freezing patterns observed in this study, it would appear that the injury occurred during freezing and not in the thawing stage. However, Hudson (26) found that a rapid thaw resulted in a greater variation in survival than a slow thaw.

Hudson and Idle (23) followed the formation of ice in the petioles of S. acaule and S. tuberosum by light microscopy and by differential thermal analysis. Their results showed that S. acaule freezes in two distinct stages, whereas the freezing process in S. tuberosum was a more continuous process. In S. acaule ice first formed in the vascular regions and gradually formed in the subhypodermal tissue. In S. tuberosum two exotherms were not evident because ice was laid down at scattered loci throughout the tissue. Sukumaran and Weiser (97) were unable to detect two exotherms during the freezing of S. acaule leaves. Perhaps this was due to the different tissues tested.

Olien (5) considers that water associated with hydrophilic plant components is critical for the survival of plant tissue during freezing. Many hydrophilic plant components such as carbohydrates,

proteins and nucleic acids tend to structure water about themselves (98). The range of this induced structure is uncertain, but Kavanau (98) suggests it will depend on the components involved. During the initial stages of freezing, water which is least affected by hydrophilic plant components has nearly a single freezing point. If the temperature is held constant, an equilibrium is established between the ice crystal and the various plant components for the remaining water. With a steadily decreasing temperature, the equilibrium is shifted in favor of the growing ice crystal. Thus the more hydrophilic components a cell has, the greater its chances for survival.

Various other plant components are known to have an effect on water. Certain small inorganic ions tend to cause the breakdown of tetrahedral water by reorientation and immobilization of water in their vicinity (99). If the concentration of these inorganic ions is high enough, a salting out of solutes will result (100). Also certain small hydrophobically hydrated ions promote water structure (99, 101). Many macromolecules, e.g., proteins and membranes, are able to structure water into so-called "icebergs" (102). This water is considered essential in maintaining the structural integrity of native macromolecules. Low temperature instability of proteins and membranes has been associated with the weakening of hydrophobic bonds at low temperatures (103, 104). Frozen membranes readily fracture in their inner hydrophobic regions due to a weakening of the hydrophobic bonds (105).

In addition to certain plant components affecting water structure, certain plant gums and xylans have a direct effect on freezing survival (106, 107). Olien (5) has termed these gums as ice kinetic inhibitors which affect frost survival by controlling the site of ice formation and the type of ice structure that develops.

An increase in membrane permeability during frost acclimation has been suggested as one means of avoiding injury (71). Hudson and Idle (23) propose that the high permeability of S.acaule cells allows solutes to readily diffuse to the extracellular ice and to initiate a thaw. This thaw accounts for the two stages of ice freezing. Sukumaran and Weiser (97) measured the water permeability of hardy S. acaule and tender S. tuberosum potatoes and found no substantial difference. Hudson and Idle (23) postulated that ice formation itself raises the permeability of the cells. Maheshwari and Sussman (108), studying cold-induced dormancy in urediospores, believe that temperature causes physical changes in the lipoprotein of cytoplasmic membranes and thus alters their

permeability. Ring (109) also found that low temperatures resulted in the widening of pores in membranes which increased their permeability. Winter wheat plants hardy to -18°C, when subjected to a frost of -5°C, lose ions at a greater rate than unfrozen controls (110). An increase in ion leakage occurred when several frost resistant potato cultivars were subjected to low non-lethal temperature (111). It is difficult to assess if this was due to increased membrane permeability or to death of a few cells which didn't affect survival.

In summary, the water content, the effect of plant components on water, patterns of initial ice growth in the tissue, the redistribution of water during freezing and the permeability of membranes are major characteristics affecting stress and are determined by genetic and environmental variables.

V. BREEDING FOR FROST RESISTANCE

Since a large number of the wild Solanum species have frost resistance, there is considerable genetic potential for incorporating this character into cultivated species (See Mastenbrock (18) and Richardson and Weiser (3) for reviews of frost resistant species). The inheritance of frost resistance is not well understood, perhaps due mainly to the lack of suitable means of screening segregating populations for frost resistance and tetrasomic inheritance. Mastenbrock (18) suggests that the genes for frost resistance are dominant and one or a few genes are involved which are quantitative or cumulative in effect. Ross and Rowe (30), working with frost-resistant diploids, reported that the F_1 progenies and $F_1 \times F_1$ progenies segregated to produce plants with a greater frost tolerance than the parental species. This would suggest that inheritance for frost tolerance is quantitative and transgressive segregation may be involved. In the same study these authors found that when the hybrids from the wild species were crossed to haploid S. tuberosum, the frequency of frost resistance in their progeny decreased approximately 50 percent. Bloomquist and Lauer (112) reported a decline in frost resistance of S. acaule x S. tuberosum hybrids and backcross derivatives as the proportion of S. acaule genes decreased. This would suggest that genes with small additive effects are involved.

S. acaule is one of the most frost resistant Solanum species (18, 26, 113, 114). Although S. acaule is a tetraploid, attempts to cross it with S. tuberosum have met with limited success (18, 115). Mastenbrock (18) partially overcame this difficulty by using a cross of two types of S. acaule. The resulting hybrids could then be crossed more readily with S. tuberosum.

Many of the frost resistant wild Solanum species are either poor yielders or do not produce tubers. This necessitates a backcross program for tuber appearance and yield. Another problem is wild species are more photoperiodic sensitive than S. tuberosum. Long days are required for flower initiation and short days for tuber production. Rudorf (115) found that after one backcross to S. tuberosum, the hybrids shifted to the behavior of S. tuberosum.

Over 60 percent of the tuber bearing Solanum species are diploid, which represent a large storehouse of relatively untapped germplasm. Attempts to form tetraploids from crosses with S. tuberosum have met with limited success. The introduction of haploids (2n = 24) of S. tuberosum L. (2n = 48) by Hougas and Peloquin (116) provided an effective means for gene transfer from tuber bearing diploids to S. tuberosum. However, the pollen fertility of many of the haploids is relatively low and thus the haploids are used as the pistillate parent. If frost tolerance of potatoes is maternally inherited as suggested by Hudson (26) this method may have limited application. The haploid, US-W4, as shown by Van Suchtelen (117), is reasonably fertile. This clone was used to pollinate other haploids and most of the plants obtained formed flowers with fertile pollen.

Van Suchtelen and Verdenius (118) crossed haploids of S. tuberosum with frost resistant diploid S. ajanhuiri and obtained hybrids which survived field frosts of -3°C.

Colchicine is widely used to induce chromosome doubling in plants. However, treatment of potatoes with colchicine frequently results in a low and variable frequency of recoverable doubled clones (119). In addition to its toxicity, mutant plants arise from colchicine treatment (120, 121). Ostergren (122) reported nitrous oxide was effective in producing polyploid Pisum sativum and Crepis capillaris if the zygotes were treated at the time of zygotic division. Nitrous oxide, applied under pressure, rapidly penetrates cells and when the pressure is released, the gas is rapidly released from the cells. This permits a finer control

of treatment in comparison to colchicine. Dvorak, Harvey and Coulman (123) found nitrous oxide to be very effective as a polyploidizing agent in barley and wheat. To the authors knowledge, nitrous oxide has not been used to produce polyploids in potatoes.

VI. THEORIES TO EXPLAIN FROST TOLERANCE

Numerous theories have been proposed to account for cold hardiness in plants. A common denominator to many is the problem of water removal from the cell during freezing. Ice crystals, growing extracellularly, cause dehydration and cellular contraction as freezing proceeds. This dehydration and contraction can lead to mechanical stress on the cell as well as concentration of the cell constituents such as proteins, salts, sugars, organic acids, etc. Described below are four theories of cold hardiness which are often cited in the literature. These thories may or may not have relevance to potato cold hardiness.

A. The sulfhydryl hypothesis - Levitt (4) proposed the sulfhydryl hypothesis. This hypothesis assumes that, because protein molecules come very close together in freeze dehydrated cells, oxidation of the sulfhydryl groups occurs on adjacent protein molecules. This oxidation results in disulfide bond formation between different protein molecules. The reaction is irreversible and thus denatures the proteins of the cell. Freezing tolerance is related to events occurring during cold acclimation which prevent disulfide bond formation.

B. The second supercooling point hypothesis - Tumanov and Krasavtsey (32) have suggested that at certain temperatures during freezing, water is restricted by the plasmalemma from moving to the extracellular ice. This results in supercooling of water in the protoplast until nucleation occurs which results in intracellular freezing. They proposed this hypothesis for plants which have low temperature exotherms.

C. The vital water exotherm hypothesis - Weiser (8) proposed the vital water hypothesis which in some ways is similar to the second supercooling point hypothesis. Weiser suggests that a certain amount of water is required by the protoplast to maintain structural integrity. During freezing a point is reached where all the readily available water has been removed by extracellular freezing. Upon

freezing, vital water is pulled away from protoplasmic constituents to the extracellular ice. This results in a chain reaction of denaturation, additional vital water loss and death.

D. The mechanical stress hypothesis - Iljin (124) proposed the mechanical stress hypothesis. He noted the collapse of cell walls which accompanied the formation and growth of ice in the intracellular spaces. On thawing of these tissues he observed that the cell wall snapped back to its original position which often tore the plasmalemma. Iljin concluded that the injury occurred only during thawing. There are certainly many exceptions to Iljin's hypothesis; however, it dramatizes the importance of controlled and slow thawing of frozen tissues.

VII. METHODS OF DETERMINING FROST TOLERANCE

A. Biological Methods - The classification of plant populations exposed to natural environments is perhaps the oldest and ultimate test for evaluating cold hardiness. However, seedling populations are exposed to the irreproducible and unreliable selection pressure of natural frost which makes results difficult to interpret. Due to microclimatological factors resulting from air drainage, difference in foliage cover and height of the plants and variation in terrain, it is difficult to obtain a uniform frost. In the case of severe frost, the entire breeding population may be lost.

Controlled freeze tests permit greater control over experimental conditions and the experiments can be replicated over time. Several parameters must be considered in using a controlled freeze test to evaluate survival. Many types, combinations and sequences of stress occur as a result of normal environmental variation. A plant may have the ability to survive one type of stress and yet not have the ability to survive others. These stresses must be recognized and duplicated in a controlled freeze test. Listed below are several artificial methods used to assess and/or predict frost tolerance. The ideal method should be quick, simple, repeatable and nondestructive. Unfortunately, to date there is no test available which meets all of these criteria.

1. Whole plant tests - This method is perhaps the simplest in preparation. Whole plants are frozen in controlled frezer chambers at a predetermined rate to a series of test-temperatures. The time

interval at which the plants are maintained at a certain test temperature varies with the researcher (18, 113). The plants to be frozen are either in flats (18) or pots (18, 113) or removed from soil (125). Upon completion of the test, the plants are thawed and then rated for injury by either regrowth (125), visual damage (113) or leakage of cell constituents (111).

Although this method appears rather simple and straightforward, there are several serious drawbacks. The researcher must have access to the plants in the chamber to initiate freezing. There must be uniform temperature distribution throughout the chamber. A variation of $\pm$ 0.5°C could give misleading results. Plants in pots with a rosette growth habit could be protected by the large heat capacity of soil. In the case when the plants are lifted and free of soil, injury may occur in the root initials. Thus a regrowth test would not be possible. In many plants such as winter cereals the root initials are killed at a warmer temperature than the foliage (126). Large variation in survival may occuer with the use of flats. Generally, those plants located at the edge of the flat show greatest injury.

Perhaps the biggest drawback in using whole plants is that the material being tested is sacrificed. Thus, a large population of plants is required which may not be possible with certain segregating populations. To ensure reproducibility of results, the test must be replicated within each test run and lines of known resistance must be included to serve as controls. The arrangement of plants must not give rise to altered air circulation and temperature variations.

In order to screen large populations, a rather large and costly freezer chamber is required. However, the larger a chamber is, the more difficult it is to maintain uniform temperature and humidity. A reliable screening test for whole plants must have very precise control of temperature throughout the chamber and the rate of temperature change must be constant and linear and at the rate desired. There should also be some way to initiate freezing of the tissue just below 0°C.

2. Cut leaf test - Although the cut leaf test is rather lengthy and requires good temperature control, it is relatively simple and non-destructive to the whole plant. According to Hudson (26), the cut leaf tests were unreliable due to irregularities in nucleation which resulted in supercooling. Hudson (26) could not distinguish resistant and non-resistant lines unless supercooling was prevented. Asahina (127) reported that sprouts from potato tubers remained supercooled for 18 hr. at -5.5° C and for four hours

at -7.5°C. If the foliage was inoculated with ice crystals at -6°C, freezing occurred intracellularly resulting in death of the cells. When the tissue sections were inoculated at -1.8°C, freezing occurred extracellularly and the tissue resisted freezing. Sukumaran and Weiser (111) nucleated their samples at -2°C on the upper surface of the leaves, whereas Hudson (26) immersed the cut end of petioles in crushed ice. According to Alien (5), nucleation usually occurs on the upper surface of leaves in the field. It is not known if the site of nucleation has an effect on the degree or type of frost injury. However, immersion of the cut end of petioles in water overnight could raise the cellular water content and have an effect on frost tolerance.

Hudson (26) concluded the main limitation of the cut leaf test was the differences in hardiness exhibited by leaves at different stages of development. To overcome this, Sukumaran and Weiser (111) selected composite samples representing leaves of different ages. Sukumaran and Weiser (111) obtained good agreement between the cut leaf test and whole plant tests. Injury was estimated quantitatively by electrolyte leakage. Various species (S. acaule, S. chomatophilum (266387 and 243340) could tolerate freezing to -5.5°C, which was in general agreement with previous reports (3). Blomquist and Lauer (112) evaluated frost resistance using the cut leaf test and found the results were consistent with observations made during natural field frosts. Although the cut leaf test is somewhat lengthy, it still appears the most reliable method of assessing frost tolerance for potatoes.

Some difficulties of the cut leaf test may be avoided by use of a temperature bar as described by Timbers and Hocking (128). A temperature bar is a flat rectangular surface (10 ft. by 2 ft.) whose temperature can be regulated as a linear gradient from one end to the other. For potato studies the temperature of such a bar would run linearly from 0°C at one end to -10°C at the other. Samples from a single test plant are distributed in uniform intervals from one end of the bar to the other. The closer samples are placed along the length of the bar the finer the hardiness determination. The bar is initially at 0°C, the samples are placed on the bar then covered by a layer of damp cheese cloth and by an insulating styrofoam cover. The cheese cloth will freeze at just below 0°C, initiating ice nucleation and preventing supercooling of the leaf tissue. The temperature gradient is then generated over a period of several hours to keep cooling rates slow. Warming rates can be controlled in a similar fashion. At the end of the experiment the samples

can be tested by visual observation for viability. Depending on the size of the bar, hundreds of samples can be tested in a period of several hours.

3. Viability tests - Visual observations of freezing injury are the quickest and simplest, but may suffer from bias. Injury to tender plants is reflected by a water-soaked appearance of the tissue upon thawing (26). However, some plants may have a water-soaked appearance initially but eventually recover. Tissue browning generally requires an incubation period of a week, but is very reliable for a range of plants (129). Regrowth is perhaps the ultimate test, but is rather lengthy (3 to 4 weeks) and considerable space is required. The test listed below are mainly objective and relatively quantitative. Not all of the tests are universal and they should be compared to regrowth tests to ensure that the test is valid.

a. Neutral red. At a neutral pH this dye is not ionized and readily enters both live and dead cells. In living cells, neutral red is ionized and retained in the cell whereas in dead cells the dye tends to readily leak out (71).

b. Conductivity method. This method is based on the amount of electrolyte which diffuses out of the cells following cold exposure (130). Electrolytes diffuse more freely from injured cells. The lethal temperature is generally regarded at the temperature when 50 percent leakage occurs. Sukumaran and Weiser (111) were able to distinguish the killing temperature of S. tuberosum and S. acaule using this method. A variation of the electrolyte conductivity method is the leakage of amino acids from cells following frost exposure (45).

c. Plasmolysis technique. Healthy cells readily plasmolyze in a hypertonic solution such as calcium chloride. When cells have been injured by freezing, the selective permeability of the membrane is lost and the cells do not plasmolyze. Microscopic examination of this tissue reveals the extent of injury and also which class of cells has been injured (5).

d. Triphenyl tetrazolium chloride test (TTC). This method is based on the reducing capacity of cells which is eventually lost after injury (131). If cells are healthy, the oxidized form of TTC, which is colorless, is reduced to the red form quickly by the cell. After an incubation period the reduced dye is extracted with 95 percent ethanol and quantified photometrically.

B. Physical Methods.

1. Nuclear magnetic resonance - Nuclear magnetic resonance (NMR) provides a very simple, quick and nondestructive way of studying tissue water. It can be used to study the status of unfrozen tissue water in terms of oriented or structured water (bound water) and it can be used to measure the amount of unfrozen water present at sub-zero temperatures. There have been several studies which have implied that bound water is involved with cold hardiness (132, 133); however, Burke et al. (134) using NMR, could not detect any evidence that bound water played a large role in cold hardiness of Cornus stolonifera. The quantity of unfrozen water between -15° and -30°C (on a dry weight basis) was not dependent on the cold hardiness of the tissue. The amount of ice formed in tender tissue is several fold higher than in hardy tissue since there is much less total water in hardy stems. A similar conclusion based on calorimetric measurements was reached for winter wheat (135). Gusta and Russell (136), using continuous wave NMR measurements detected a broadening of the NMR absorption band for water during cold acclimation of winter wheat. During deacclimation there was a reversal in line width. NMR will certainly be of use in understanding the dynamic and freezing properties of tissue water. There are two methods of NMR, continuous wave NMR and pulsed NMR. In both of these methods, a sample is placed in a test tube (0.3 to 1.0 cm in diameter) and inserted into the instrument. Besides the fact that the tissue must be excised, the method is non-destructive to the tissue.

Continuous wave NMR is radiowave absorption spectroscopy requiring a magnetic field and depending on the strength of magnetic field used, resonance of protons occurs between 10 mHz to 330 mHz (commercially available spectrometers). There are two types of continuous wave spectrometers. In the most common type, the radiowave light source is held at a constant frequency and the magnetic field strength is varied. At certain field strengths, resonance occurs and at resonance an absorbance line is obtained. Alternatively in the second type of spectrometer, the magnetic field intensity is held constant and the radiowave frequency of the light source is varied slowly through the resonance range.

The NMR spectrum is a plot of absorbance on the ordinate vs frequency and/or magnetic field intensity on the abcissa (Fig. 1). integrated absorption intensity (area under the absorption curves) is proportional to the liquid water content of the sample (Equation 1). In this equation, L equals the total water present (percentage of fresh weight), L equals the liquid

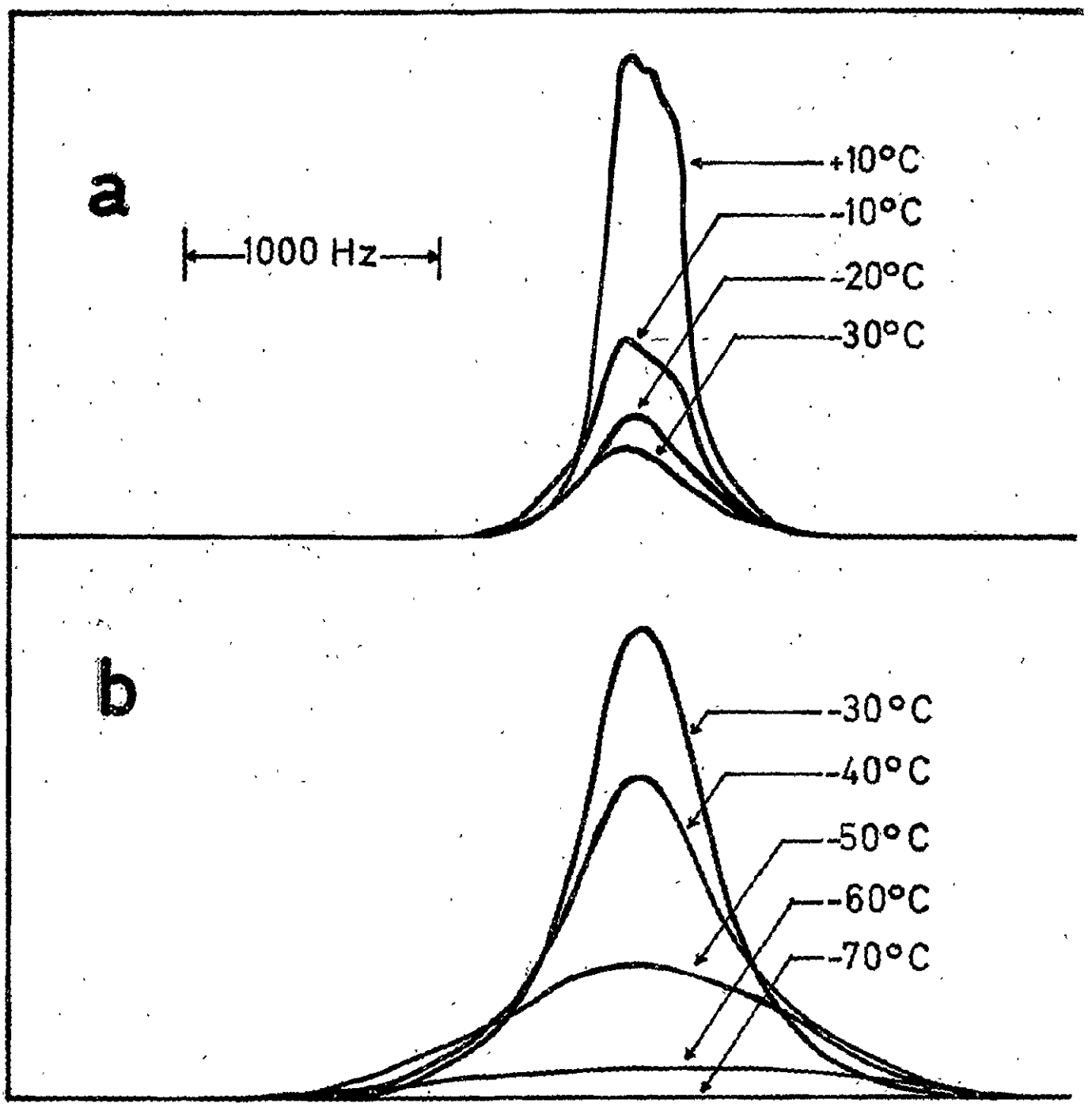

Figure 1. 100 MHz NMR spectra of an acclimated dogwood stem. a) Spectra between + 10C a -30C. b) Spectra between -30C and -70C. In b the radio frequency field strength wa increased by approximately 13-fold which increased the sensitivity. The cylindrical sample was stationary and perpendicular to the external magnetic field.

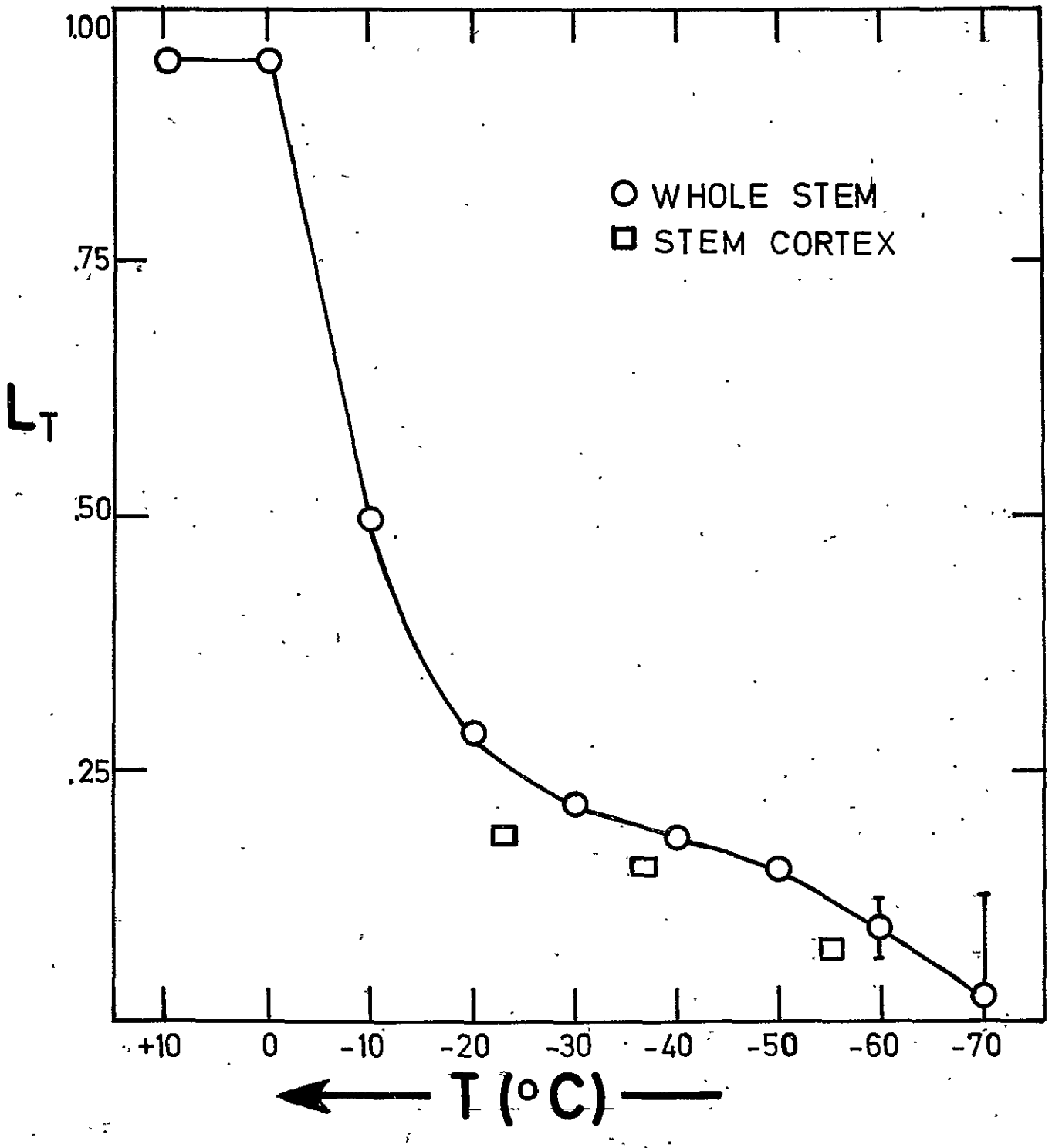

Figure 2. Freezing curve of hardy dogwood stem and hardy dogwood bark. The data were obtained by integrating 40000 Hz of the continuous wave NMR spectra at 100 MHz. L_T is defined in Equation 1 and is expressed in grams liquid water per gram dry sample. The spectra used are those in Figure 1. The full band width never exceeded 8000 Hz.

water present at temperature T (total water - ice), A_{10}^{t} equals the integrated absorption intensity at 10°C, A_t equals the integrated absorption intensity at temperature T, and T equals temperature in °K.

$$L = L_t \frac{A_t}{A_{10}} \frac{283}{T}$$

In Fig. 1 examples of an NMR absorption spectrum (a) and the freezing curve (b) obtained from such spectra are given.

In Fig. 2,the absorption line becomes broader as the temperature is reduced, thus making integration of the curve progressively more difficult. The line width is proportional to the rotational correlation for water (an expression of the ease of rotation of water molecules) and therefore potentially provides information on the amount of "bound" water in the plant tissue; however, attempting to attribute line width solely to "bound" water content of the tissue is subject to considerable error. Not the least difficulty arises from defining the term "bound" water. Some of the problems of line width interpretations can be avoided by the use of pulsed NMR.

In contrast to continuous wave NMR, both the radiowave frequency of the light source and the magnetic field intensity are adjusted in pulsed NMR spectroscopy. In the experiment the sample which is in the magnetic field of the spectrometer is exposed to a brief pulse (usually less than 10 μsec) of radio frequency light. The sample absorbs the light, goes into an excited state which is metastable and decays with time. Because the decay time is short, the signals are monitored on an oscilliscope. Two kinds of pulses are commonly used, 90° and 180° pulses. In the magnetic field of a pulse spectrometer the protons are predominantly aligned with the magnetic field of the spectrometer. After a 90° pulse, protons are oriented perpendicular to the magnetic field of the spectrometer. After a 180° pulse, the protons are oriented antiparallel (against) the magnetic field. The electronics of the spectrometer are such that only protons which are oriented perpendicular can be observed, therefore, following a 90° pulse a signal is observed which decays with time. The decay process after a 90° pulse is called free induction decay. After a 180° pulse no signal is observed although the direction of the protons orientation is changed.

Two relaxation times are commonly measured on the pulsed spectrometer. They are spin-spin (T_2) and spin-lattice (T_1) relaxation times. These relaxation times provide a measure of the dynamic properties of the tissue water such as the fraction of water bound, its viscosity and self-diffusion coefficient. Combinations of 90° and 180° pulses are needed for these measurements. Good reviews of these relaxation processes and their interpretation can be found elsewhere (137). The half life of the free induction decay of ice and most nonaqueous components of biological tissues is very short, usually less than 10 μsec. Conversely, liquid water has a longer half life (milliseconds to seconds). Therefore, if one monitors the free induction decay 15 μsec after the 90° pulse, the only signal remaining results from the liquid water. That signal is analogous to the integrated absorption intensity, A_t, in equation 1. This output signal, A_t, can be fed into a recorder and the liquid water content can then be recorded directly as the sample is cooled or warmed.

2. Thermal Analysis - The freezing of water is an exothermic reaction. This property of water has been used by several researchers to determine at what temperatures substantial amounts of water freeze in tissues and if any of the observed exotherms are related to injury (23, 138-142). Heat which is released by the crystallization of water can be measured by calorimetry, fine thermocouples, etc.

Exotherm studies have several advantages in that they are relatively quick, only small samples are required and the results are available in 2 to 6 hours. Unfortunately, only in a few instances has it been possible to demonstrate that exothermic changes are associated with freezing injury to hardy plants. Tumanov and Krasavtsev (138) using calorimetric and microscopic studies during freezing of stems, demonstrated a calorimetric lag in the freezing processes at temperatures slightly above the killing point and a discrete release of heat and a loss of fluorescence in cells at the moment of injury. Graham (141) working with hardy deciduous azalea buds reported a distinct exotherm which was associated with injury. Quamme, Weiser and Stushnoff (142) using differential thermal analysis found an exotherm at low temperature which was associated with injury to the xylem and pith. Hudson and Idle (23) were able to detect two distinct exotherms during slow freezing of S. acaule petioles. In S. tuberosum these two exotherms were less well defined. By following the process of freezing by light microscopy in S. tuberosum, Hudson and Idle (23) were able to relate the exotherms to the pattern of freezing in the tissue. S. acaule

first froze in the vascular tissues and then in the adjacent extracellular spaces whereas in S. tuberosum ice forms at scattered sites throughout the tissue. Sukumaran and Weiser (97) working with the same two species could only detect one exotherm in their thermal differential freezing profiles. In this study the thermo junctions were affixed to the leaf surface whereas Hudson and Idle (23) had their thermo junction inserted in the petiole.

Three methods are used for thermal analysis, 1) thermal analysis as such, 2) differential thermal analysis and 3) differential scanning calorimetry. These methods are used to determine the freezing and thawing points of tissue water. Differential scanning calorimetry is also used to estimate the fraction of water frozen.

The equipment required for thermal analysis includes a thermocouple for sample temperature measurement, a temperature recorder and a device to cool the samples at a controllable rate. This method is used to show the freezing point of leaf tissue and also to make a qualitative estimate of rate of tissue water freezing.

Differential thermal analysis differs from thermal analysis in that both a reference sample of known thermal characteristics and the sample are cooled simultaneously and the temperature difference between the two is recorded. This is usually performed using two thermocouples in series, one in the reference and one in the sample. Data from differential thermal analysis is generally plotted as the temperature difference between sample and reference on the ordinate vs sample temperature, reference temperature or time on the abscissa. This technique is applied in the same fashion as thermal analysis; however, the differential method is more sensitive. Both methods provide only a qualitative estimate of the quantity of frozen water at various temperatures. The chief advantage of these methods is the simplicity of the equipment necessary for the experiment.

Differential scanning calorimetry has been applied to cold hardiness research, and has employed two types of calorimeters, the Calvet calorimeter used by Krasavtsev and Olien and the more conventional scanning calorimeter such as the Perkin-Elmer, Dupont, Stone, etc. These instruments perform the same tasks as thermal or differential thermal analysis instruments do with the added feature of determining the amount of water frozen or thawed between any two temperatures. Unlike the other thermal analysis methods which measure temperature or temperature difference, the differential scanning calorimeter measures the difference in the heat evolved or absorbed during cooling or warming between an unknown sample and known reference. The reference varies from air to metals depending on the

sample requirements. The amount of water frozen can be determined from the heat evolved during cooling using the heat of fusion of water and heat capacities of ice and liquid water. The main weakness in calorimetric measurements is in the choosing of heats of fusion and heat capacities for tissue water, particularly for the liquid water remaining at low temperature in a partially frozen tissue. For potato foliage where interest is focused in the 0° to 10°C temperature range, these above difficulties are probably minor. Another factor making any error from the choosing of these terms minor in potato studies is that any study of potato will be a comparative study between the hardy and tender species. Thus, the magnitude of errors introduced by incorrect heat capacities or heats of fusion will tend to be attenuated so long as comparison measurements are used.

3. Electrical resistance - Electrical measurements have been shown to be a useful procedure for the evaluation of frost hardiness in certain plant species (20, 143-145). In 1931, Luyet found a decrease in electrical impedance of plants after the tissue had been injured by cold, heat or lipid solvents (146). Luyet attributed the decrease in electrical impedance observed at low frequencies to the degree of destruction. Greenham and Daday (147) working with white clover and alfalfa, attributed the drop in electrical impedance following cold injury to the destruction of the plasmalemma.

Electrical measurements on woody tissue to assess winter hardiness have had varying degrees of success. Weaver et al. (148), working with peach scions, could not separate different hardiness groups. Also the method was restricted to bearing trees. Craig, Gass and Fensom (149) were unable to differentiate between winter hardy and tender cultivars of raspberry due to extensive physiological changes occurring during development. Hayden, Dionne and Fensom (20) reported that electrical impedance measurements of the stems or petioles of Solanum clones were a reliable method of assessing relative frost hardiness. However, in order to make valid comparisons between clones, the plants had to be grown and tested under carefully controlled conditions and at least 10 plants were required for averaging.

In the studies discussed above, most of the electrical measurements were done at a single low frequency and without temperature control. Measurements at low frequencies are influenced by membrane changes (145) and changes in stem diameter (148), electrolyte concentration (146), cell size (147) and changes in temperature (150). To minimize these variables Evert and Weiser (145)

used a ratio of a high and a low frequency impedance for predicting the cold hardiness of stem sections of red-osier dogwood.

Electrical impedance measurements offer practical advantages over the previous techniques because they are determined with ease without excising the tissue. Impedance with alternating current is analogous to resistance with direct current. In contrast to previous methods which provide information on the freezing water, electrical impedance measurements provide information on the integrity of membranes, cell walls, etc. in the tissue since electrical impedance decreases when membrane destruction occurs.

Impedance measurements are performed by inserting electrodes into the tissue to be studied and the impedance is measured directly. Certain assumptions must be made in interpreting the results of electrical impedance. The assumption is that biological systems act analogous to an electrical model. In the model the membrane provides a source of capacitance. The destruction of the membrane will produce an effect analogous to the loss of capacitance in the electrical circuit. More sophisticated models can be employed, but the simple model seems to be satisfactory for most studies.

VIII. COLD RESISTANCE PROJECT PLANNING CONFERENCE OBJECTIVES

The long term objective is to develop high yielding and high quality potatoes with improved frost tolerance. Initial primary emphasis should be placed on: 1) Selection of the most suitable screening technique considering the limitation of personnel and facilities, and 2) Initiate a potato breeding program involving recombinations between S. tuberosum and frost tolerant species.

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APPENDIX II

BREEDING POTATOES FOR FROST TOLERANCE

N. Estrada Ramos

1. Genetic sources

At least sixteen potato wild species have been reported as having high frost tolerance. The following can be mentioned: S. acaule, boliviense, brevicaulae, Bukasovii, chomatophilum, canasense, Commersonii, demissum, multidissectum, megistacrolobum, sanctae-rosae, sogarandinum, toralapanum, tuquerrense, vernei.

High tolerance has also been reported in some clones of the five cultivated species, S. andigena (tuberosum), curtilobum, Juzepczukii, ajanhuiri and stenotonum.

Resistance is then found in all ploidy levels of the known potato species (2n = 24, 36, 48, 60, 72), and indicates that there is a good genetic potential to breed cultivated potatoes with frost tolerance.

Different degrees of resistance have been found in various species and clones but a general division could be made into 3 groups. The first or high resistance group would include probably, S. acaule, S. chomatophilum, S. etuberosum, S. Commersonii. The second group or next in resistance may include S. Juzepczukii, S. ajanhuiri, demissum, multidissectum and most of the wild frost resistant species.

The third group will include the cultivated species S. curtilobum, stenotomum, andigena. Firbas and Ross (1961), Ross and Rowe (1969), Estrada (1953), Hawkes (1958).

2. Crossability

Crossability has not been a barrier to improve resistance since hybrids between resistant species and good cultivated potato clones have been reported frequently in the literature (Bukasov, Kameraz, Mastenbroek, Blanco and Ubeda, Blomquist and Lauer, Ross and Rowe, Estrada, etc.).

There are difficulties in crossing some of the most resistant as S. etuberosum and S. chomatophilum, but others like S. acaule, brevicaule, multidissectum, ajanhuiri, Bukasovii may be crossed rather easily,

The crosses using the resistant clones of cultivated species are easy with exception of S. Juzepczukii which is highly sterile because it is a triploid, interspecific hybrid, which brings much chromosome imbalance.

3. Heritability

Resistance is reported to be inherited in many cases as a dominant factor, especially in crosses using S. acaule (Mastenbroek 1956, Blanco and Ubeda 1966).

In other cases it is reported as a cumulative or as a recessive character.

Vesselovskii (Hudson 1936) obtained acaule x goniocalyx hybrids which survived -5.5°C and could be crossed to S. tuberosum. Hybrids between diploid cultivated species and S. brevicaule, S. vernei and S. multidissectum have been reported resistant to -5°C by Richardson and Estrada (1971). Good resistance was also found by Blomquist and Lauer (1962) in acaule x tuberosum hybrids and by Blanco and Ubeda (1966) in acaule x tuberosum hybrids.

Gene exchange

Blanco and Ubeda (1966) found that backcrossing to tuberosum employing S. acaule as a frost resistant source showed resistance to -5°C for 3 hours and relatively high tuber yield. This was confirmed by Estrada (1965) and both authors were able to observe quadrivalents in the hybrids (F_1) which suggested good possibilities of gene exchange.

The heriability of frost resistance in the hybrids appears to be good according to reports on resistance given by Bukasov, Kameraz, Mastenbroek, Estrada, etc. They have found little linkage between frost reaction and undesirable characters and good tuber type plants can be obtained in the first backcrosses, Estrada (1965).

Inheritance patterns, however, in advanced generations appear complicated. They could be simplified using diploid species according to Ross and Rowe (1965). As far as obtaining varieties with frost resistance, only a limited degree of resistance has been incorporated. Dearborne (1967) Estrada et al (1972).

4. Selection systems

Different authors have used various systems to test for resistance. Firbas and Ross (1961) used detached leaves and left seedlings in gauze frames exposed to -2.5°C for 2 hours. Blomquist and Lauer (1962) used detached leaves and Blanco and Ubeda (1966) used the whole plants in pots. Ross and Rowe (1969) submitted the plants to natural frost conditions in the field (at the end of fall) and Richardson and Estrada (1971) and Alvarado et al (1972) tested plants in growth cabinets and in the field under natural occurring frosts.

Different selection methods thus far used have resulted in some progress but they are still the main limiting factor in a mass program of testing. The main problem is to develop a system as close to natural conditions as possible, and to obtain precise temperature control. Otherwise a mechanism for testing the influence of potentially injurious cold stress without subjecting the plant to cold stress would be useful.

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